Supplementary material for "CD4/CD8 ratio and CD8 counts predict CD4 response in HIV-1 infected drug naive and in patients on cART"

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1 Linear mixed model for longitudinal SHCS data

The SHCS data is collected for patients $i = 1, \ldots, m$ who are observed repeatedly at follow-up visits $j = 1, \ldots, n_i$ at times t_{i1}, t_{i2}, t_{ij}, where $j = 1, \ldots, n_i$. The square root transformed CD4 cell counts is the outcome $\sqrt{\text{CD4}_{ij}} = y_{ij}$ which is assumed to follow a normal distribution. The observations for a single patient i are modeled by a linear predictor

$$\mathbf{y}_i = \mathbf{x}_i\boldsymbol{\beta} + \mathbf{z}_i\mathbf{b}_i + \boldsymbol{\epsilon}_i.$$

The observations for this patient are collected by the vector $\mathbf{y}_i$ of length n_i. The p different fixed effect covariates are in the design matrix $\mathbf{x}_i$ of dimension $(n_i \times p)$. The coefficients for the fixed effect predictors are in the vector $\boldsymbol{\beta}$ and is of length p. The

patient-specific random effects vector $\mathbf{b}_i$ is usually a subvector of $\boldsymbol{\beta}$ with length $q < p$ and is multiplied by the random effects design matrix $\mathbf{z}_i$ of dimension $(n_i \times q)$.

The linear mixed model assumes that the residuals follow a normal distribution $\boldsymbol{\epsilon}_i \sim \mathcal{N}(\mathbf{0}, \sigma^2_\epsilon)$ and the errors are independent and identical distributed. The patient-specific random effects $\mathbf{b}_i$ are also assumed to follow a normal distribution $\mathbf{b}_i \sim \mathcal{N}_q(\mathbf{0}, \mathbf{D})$. In the case of random intercept and random slope $q = 2$ and the random effects covariance matrix $\mathbf{D}$ is of dimension 2×2. Further the error term $\boldsymbol{\epsilon}_i$ and the random effects $\mathbf{b}_i$ are assumed to be independent. The patient-specific marginal likelihood integrates over the random effects $\mathbf{b}_i$ and follows a multivariate normal distribution with mean $\mathbf{x}_i\boldsymbol{\beta}$ and covariance matrix $\mathbf{z}_i\mathbf{D}\mathbf{z}_i^\top + \mathbf{I}_{n_i}$, where here $\mathbf{I}_{n_i}$ is the identity matrix of dimension n_i.

Model M1, described in the main text, included the following covariates for which a fixed effect coefficient was estimated:
$\mathbf{x}_{ij} = (1, t_{ij}, \sqrt{\text{CD4}_{i,(j-1)}}, \log(\text{RNA}_{i,(j-1)}), \text{AIDS}_{ij}, \text{age}_{ij}, \text{transmission}_i, \text{HCV}_i)$ and in the cART group additionally the effect for NRTI_i. Model M2 estimated the same fixed effects as M1 but additionally included $\sqrt{\text{CD8}_{i,(j-1)}}$. Model M3 estimated the same fixed effects as M1 but additionally included $\log(\text{CD4}_{i,(j-1)}/\text{CD8}_{i,(j-1)})$. The random effects structure is the same in all models and included a patient-specific random intercept and random slope such that $q = 2$ and the design matrix $\mathbf{z}_{ij} = (1, t_{ij})$.

1.1 Model choice criteria

Different models with different predictors can be compared by model choice criteria such as the AIC or BIC. Common model selection criteria, like AIC or BIC, are not applicable to linear mixed models. The main reason for this is the difficulty to assess the degree of freedom for the random effects included in a linear mixed model. Different proposals for extensions of the common model choice criteria to linear mixed models exist. One suggestion for a model choice criteria to mixed models with different fixed effects but the same random effects structure is the modified BIC [1], who defines the version of the BIC, modified for linear mixed models as

$$\text{BIC} = -2\log L + \sum_{k=1}^{p} \log(n_k)$$

where L is the likelihood and n_k is equal to the number of individuals I if the predictor is included as fixed and as random effect, or the number of observations $n = \sum_{i=1}^{I} n_i$ if the predictor is included as fixed effect only. See also [2] for a description of model choice criteria and about predictive comparisons for generalized linear mixed models.

1.2 Explained variation

A goodness of fit criteria for linear regression models is the R^2, which is a measure for the variation expressed by the model in relation to the overall variation in the data. In the case of a multiple regression model the R^2 needs to be adjusted for the number of included parameters, in order to gain comparability across different models with different parameters. For linear mixed models one needs to adapt the R^2 additionally for the variation explained by the random effects, which can be done in different ways.

Properties for a sensible R^2 measure are discussed by [3], who suggest a R^2 measure for linear mixed models with random intercepts. They distinguish between the marginal R^2, which expresses the variance explained by fixed effects as proportion of all variance components and the conditional R^2, which is a measure for the variance explained by fixed and random effects. This idea was extended to linear mixed models with random intercepts and random slopes by [4].

According to equation 29 in [3] the marginal R^2 for a random intercept model is defined as

$$R^2_{\text{marignal}} = \frac{\sigma_f^2}{\sigma_f^2 + \sum_{l=1}^{q} \sigma_l^2 + \sigma_\epsilon^2}$$

where σ_f^2 is the variance attributable to the fixed effects, σ_l^2 is the variance of the lth of the q random effects. For the extension to random intercepts and random slopes [4] proposes to replace $\sum_{l=1}^{q} \sigma_l^2$ by the mean random effect variance

$$\bar{\sigma}^2 = \text{tr}(\mathbf{z}\mathbf{D}\mathbf{z}^\top)/n$$

where n is the number of observations ($n = \sum_{i=1}^{I} n_i$) and $\mathbf{z}$ the random effects design matrix for all patients of dimension $n \times 2$.

1.3 Time course for NAIVE and cART

In this section we illustrate in more detail how the applied linear mixed model for longitudinal data takes the time trend since cohort entry (NAIVE) or since therapy start (cART) into account. The time trend for both subgroups is illustrated in the upper two plots in Figure 1 which is based on the model M3 presented in the main text but would look rather similar for model M2. For Figure 1 all covariates are set to its average values and held constant and categorical variables are set to the reference category, while only the time since cohort entry for NAIVE or since therapy start for cART is increasing.

The fixed effect time trend, representing the population mean of the square root transformed CD4 course and given the reference categories for the transmission and the HCV factors, is plotted as black line. The dark grey area around the global fixed time effect shows a 95% confidence interval (CI) for the average population time trend. The light grey area shows a 95% CI for the time-dependent prediction error based on the standard deviation of the model residuals (σ_ϵ) and the standard deviation of the fixed time effect (σ_f). This prediction error band is based on the artificial data used for the plot, for which time since cohort entry (NAIVE) or since therapy start (cART) is the only varying covariate.

The linear decreasing time trend for NAIVE is opposed by a sharply increasing time trend in cART, as the time scale was square root transformed for the cART subgroup. This reflects that CD4 cells for untreated HIV infected patients are steadily declining where on average the CD4 level is recovering after cART initiation.

The dashed lines in the upper two plots of Figure 1 show patient-specific deviations from the population mean based on random intercepts and random slopes of 20 different, randomly sampled patients. These individual intercepts and time courses reflect how the linear mixed model takes the between patient variation at baseline and during disease progression into account. The model yields a rather high flexibility to cover individual time courses, especially just after cART initiation: a positive random slope on the square root transformed time scale leads to a sharp but flattening increase in CD4, especially if the CD4 level was already impaired which is reflected by a relatively small random intercept. A negative random slope instead is often present among the cART group if the CD4 level at therapy start was still high. A negative random slope implies a flattening decline in CD4 cell counts after cART initiation.

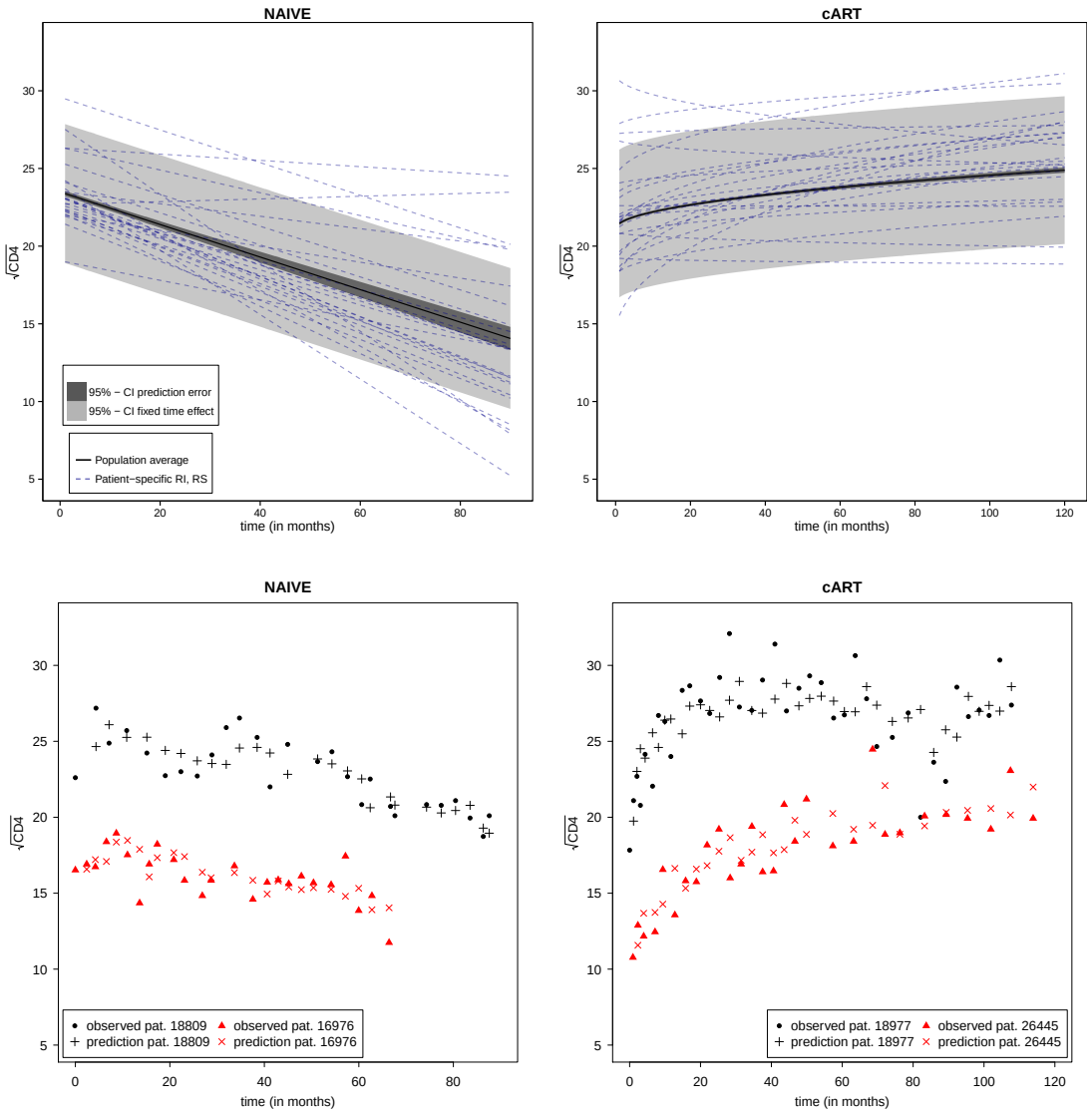

Figure 1: Profiles with time trend in the NAIVE and cART based on model M3 for the population average and 20 randomly selected patients (pat.) in the upper two plots. Observed values and model predictions for two selected patients in each group in the lower two plots.

The lower two plots in Figure 1 show observed square root transformed CD4 cell counts obtained from two NAIVE and two cART patients, as well as each correspond-

ing model prediction for disease progression. The four patients in the lower plots are chosen such that their response values do not overlap and that they have a substantial record of observed lymphocytes. The lower two plots serve for illustration purposes only and should clarify how the model applies to the patient-specific longitudinal data.

1.4 Random effect structure and residual analysis

The parameter estimates of the random effect covariance matrix ($\hat{\mathbf{D}}$) are shown in Table 1 based on model M2 and model M3, presented in the main text, together with the residual standard deviations ($\hat{\sigma}_\epsilon$). The estimated random effect structure and the standard deviation for the residuals are very similar for both models. From Table 1 we see that the patient-specific variation of the intercept has about the same magnitude as the residual standard deviation. The size of the residual standard deviation for model M3 is also visualized in Figure 1 in the 95% prediction error band as light grey area.

	NAIVE		**cART**	
	M2	**M3**	**M2**	**M3**
RI Stdev.	2.272	2.267	3.330	3.130
RS Stdev.	0.093	0.090	0.517	0.502
correlation	-0.260	-0.309	-0.685	-0.646
Resid. Stdev.	2.281	2.280	2.430	2.425

Table 1: Estimated random effect structure and residual standard error of linear mixed models M2 and M3 for each patient subgroup (NAIVE and cART).

Figure 2 shows a QQ-plot of the residuals for models M2 and M3 and the NAIVE and cART patient groups. The residuals in Figure 2 and 3 are raw residuals divided by the corresponding standard errors and further normalized by the inverse square-root factor of the estimated error correlation matrix (see `residuals.lme` in the R-package `nlme` for more information).

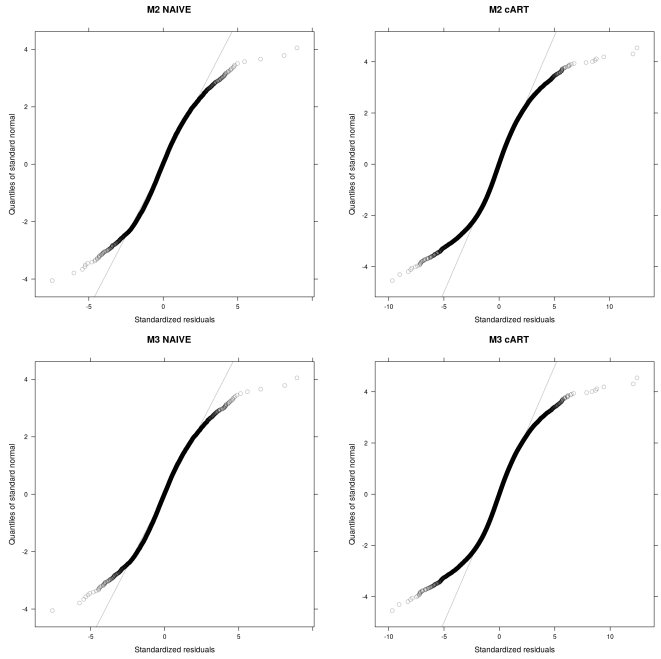

Figure 2: QQ-plots with standardized reisduals in model M2, M3 for NAIVE, cART.

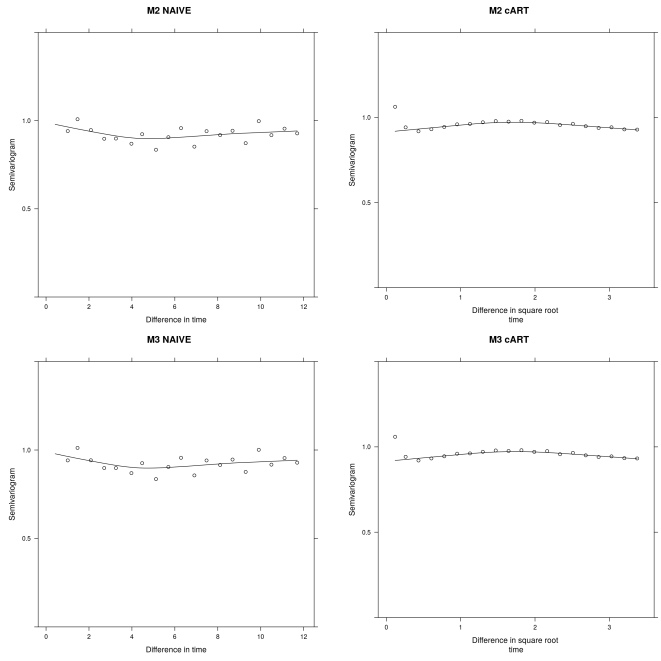

Figure 3: Variogram with standardized reisduals in model M2, M3 for NAIVE, cART.

2 Effect size of predictors

To overcome the difficulties in giving an interpretation of the model coefficients, which are transformed to different non-linear scales, we illustrate the effect sizes of the predictors and the transformation from $\sqrt{\text{CD4}}$ to original CD4 cell counts with a plots in Figure 4 and 5 for model M2 and in Figure 6 and 7 for model M3. The horizontal bars indicate the lower 2.5% and the upper 97.5% quantile as well as the median (black dot) of each covariate multiplied by its coefficient estimate, labelled with the corresponding quantiles of the covariates. As the level effect sizes for the categorical predictors HCV and transmission are very small, they are omitted in Figure 4 to 7.

The contribution of each predictor to the square root transformed response (here $\sqrt{\text{CD4}}$) is additive and can be read off for every predictor from the line at the bottom (predicted $\sqrt{\text{CD4}}$). Summing up each contribution to the response for all predictors gives the prediction of the square root transformed CD4 cell counts at the next follow-up visit. The vertical scale on the right allows to translate from the predicted square root scale (small figures) to the CD4 cell counts (large figures). On the right vertical scale also the fixed effect intercept, which must be added to the predicted value, is indicated by a black dot. The error bar for the fixed effect intercept is representing a 95% CI based on the estimated random intercept standard deviation. One can read off from the scale for the absolute CD4 cell counts on the right, that the same difference in the linear predictor causes a larger difference in the absolute CD4 cell count, if the predicted square root CD4 cell count is higher.

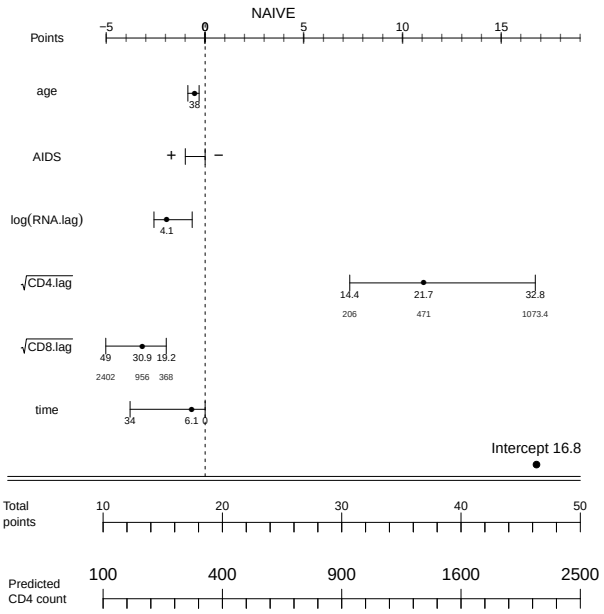

Figure 4: Effect size of predictors for NAIVE patients and model M2.

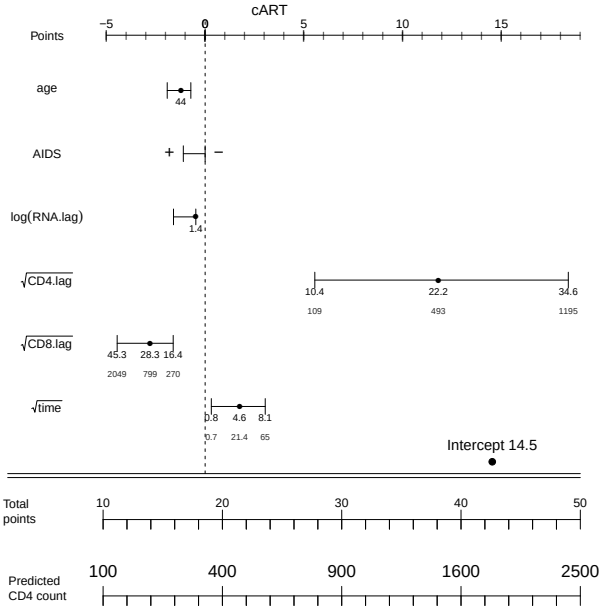

Figure 5: Effect size of predictors for cART patients and model M2.

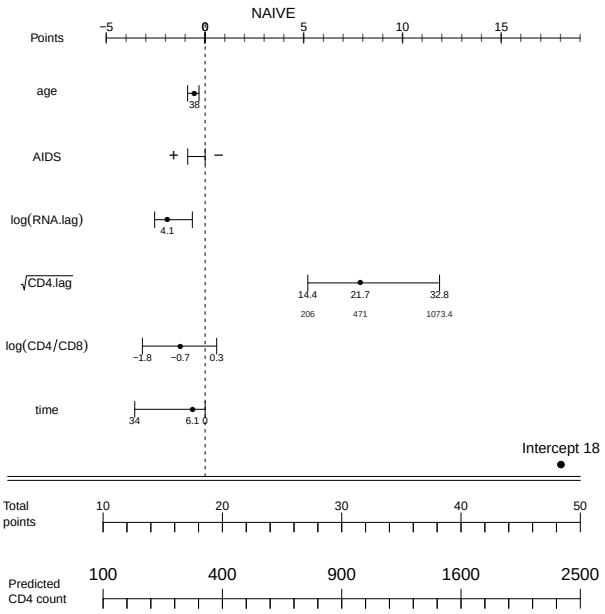

Figure 6: Effect size of predictors for NAIVE patients and model M3.

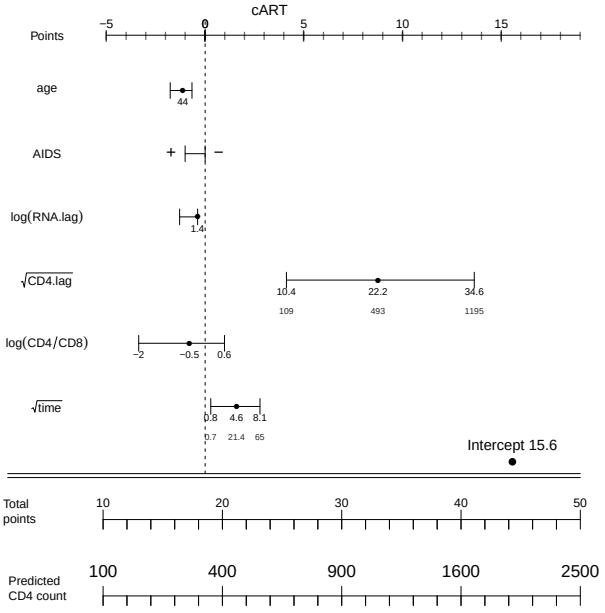

Figure 7: Effect size of predictors for cART patients and model M3.

3 Coefficient estimates for Model M2

Table 2 shows the coefficient estimates for the longitudinal predictors for model M2, corresponding to Table 3 in the main text, which reported the estimates for model M3. Equivalently Table 3 shows the coefficient estimates for the additional coefficients based on Model M2, corresponding to Table 4 in the main text.

	NAIVE		**cART**	
	coef.	95% - CI	coef.	95% - CI
intercept	16.78	16.21 to 17.36	14.54	14.30 to 14.78
time	-0.11	-0.12 to -0.10		
square root time			0.38	0.36 to 0.40
CD4	0.51	0.50 to 0.52	0.53	0.53 to 0.54
RNA	-0.47	-0.53 to -0.40	-0.34	-0.36 to -0.32
CD8	-0.10	-0.11 to -0.09	-0.10	-0.10 to -0.09

Table 2: Coefficient estimates (coef.) with 95% confidence intervals (CI) for longitudinal predictors and both patient subgroups (NAIVE and cART) for model M2. All p-values are <0.0001. Estimates for additional predictors are in Table 3.

	NAIVE			**cART**		
	coef.	95% CI	p-value	coef.	95% CI	p-value
AIDS at follow-up visit	-1.00	-1.41 to -0.60	< 0.0001	-1.10	-1.21 to -0.99	< 0.0001
age at follow-up visit	-0.01	-0.02 to 0.00	0.0023	-0.03	-0.03 to -0.02	< 0.0001
NRTI at baseline				-0.02	-0.02 to -0.01	< 0.0001
transmission			0.01			< 0.0001
MSM (reference)	0.00			0.00		
IDU-male	-0.13	-0.60 to 0.34		-1.19	-1.45 to -0.93	
IDU-female	-0.11	-0.70 to 0.49		-0.73	-1.04 to -0.42	
HET-male	-0.35	-0.63 to -0.07		-0.64	-0.79 to -0.48	
HET-female	-0.40	-0.65 to -0.14		-0.44	-0.58 to -0.29	
other	-0.07	-0.58 to 0.43		-0.34	-0.61 to -0.08	
HCV			0.39			< 0.0001
negative (reference)	0.00			0.00		
inactive	-0.09	-0.61 to 0.42		0.02	-0.28 to 0.33	
active	-0.16	-0.52 to 0.21		-0.45	-0.66 to -0.24	

Table 3: Estimates for additional predictors for model M2.

4 Time spans between follow-up visits

Figure 8 shows a histogram for the time between two subsequent follow-up visits for the NAIVE and the cART subgroups. The vertical dashed line indicates a follow-up time of 12 months. All observations with a follow-up time of more than one year were censored.

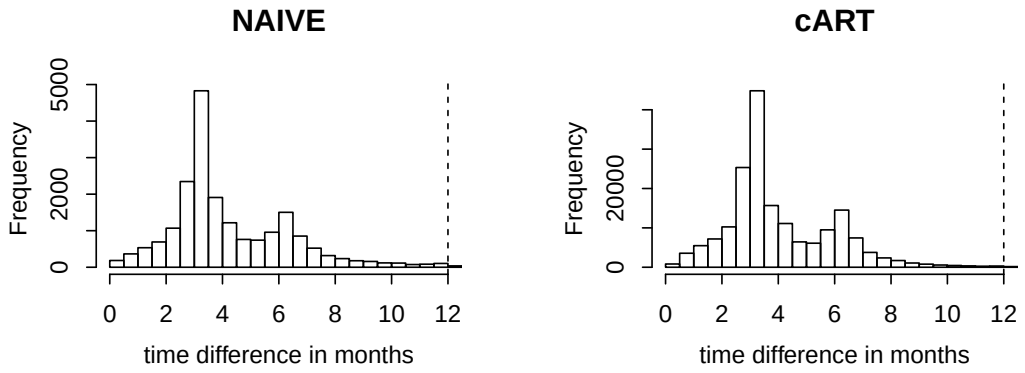

Figure 8: Histograms for time differences between two subsequent follow-up visits in months. Time is equal to zero at cohort entry for NAIVE and at therapy initiation for cART.

References

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